ORIGINAL ARTICLE

Asian Herpetological Research 2022, 13(2): 109–116
DOI: 10.16373/j.cnki.ahr.210065

A New Species of *Nidirana* (Anura, Ranidae) from Southern Guangxi, China

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Abstract A new music frog, *Nidirana shiwandashanensis* **sp. nov.**, is described from Shiwandashan National Nature Reserve, Shangsi County, Guangxi, China. Phylogenetic analysis based on mtDNA sequences indicates that the new species is closest to *N. guangxiensis*. However, *N. shiwandashanensis* **sp. nov.** differs from its congeners based on morphological characters, molecular data, and bioacoustics. The advertisement calls are comprised of 6–8 double notes, which are distinct from its sister taxon, *N. guangxiensis*. Currently, the new species occurs in the evergreen forest at Shiwandashan National Nature Reserve between 500–600 m a.s.l.

Keywords bioacoustics, morphology, *Nidirana*, phylogeny

1. Introduction

The genus *Nidirana* Dubois, 1992 is distributed in eastern and southeastern Asia (AmphibiaChina, 2021; Frost, 2021). For a long time *Nidirana* has been regarded as a subgenus of *Rana* Linnaeus, 1758. Based on large-scale species sampling and comprehensive morphology, molecular analysis, and bioacoustics data, Lyu *et al.* (2017) suggested that the genus *Nidirana* is a distinct genus and a sister taxon to the genus *Babina*. In recent years, *Nidirana* species diversity increased dramatically from 7 to 16 (AmphibiaChina, 2021; Frost, 2021), namely *N. guangdongensis* Lyu, Wan and Wang, 2020; *N. guangxiensis* Mo, Lyu, Huang, Liao and Wang, 2021; *N. leishanensis* Li, Wei, Xu, Cui, Fei, Jiang, Liu and Wang, 2019; *N. mangveni* Lyu, Qi and Wang, 2020; *N. nankunensis* Lyu, Zeng, Wang, Lin, Liu and Wang, 2017; *N. occidentalis* Lyu, Yang and Wang, 2020; *N. xiangica* Lyu and Wang, 2020; *N. yaoica* Lyu, Mo, Wan, Li, Pang and Wang, 2019; and *N. yeae* Wei, Li, Liu, Cheng, Xu and Wang, 2020. This high frequency of new species descriptions indicates that the species diversity of *Nidirana* was and most like still is substantially underestimated.

Due to conservative phenotypes, *Nidirana* species from southern China were often treated as *Nidirana adenopleura* (Lyu *et al.*, 2017, 2019, 2020a, 2020b). Currently, there are four recognized *Nidirana* species in Guangxi, i.e.: *N. guangxiensis, N. leishanensis, N. xiangica,* and *N. yaoica* (AmphibiaChina, 2021; Lyu *et al.*, 2021), with *N. guangxiensis* and *N. yaoica* having usually been identified as *N. adenopleura* (Lyu *et al.*, 2019, 2021). In 2021, we surveyed Shiwandashan National Nature Reserve (SWDS), Shangsi County, Guangxi, China, and collected seven *Nidirana* specimens. After analyzing the specimens based on morphology, phylogeny, and bioacoustics, we found that these specimens differed from the known *Nidirana* species. Herein, we describe the specimens as a new species of *Nidirana*.

2. Materials and Methods

2.1. Specimens Four adults, one sub-adult, and two tadpoles were collected in SWDS. All specimens were fixed in 10% formalin for 48 h, then transferred to 75% ethanol, finally deposited in Nanning Normal University. Muscle samples were stored in 100% ethanol for molecular analysis.

2.2. Morphological examination The definition of morphological characters refers to Lyu *et al.* (2021). The following measurements were taken with digital calipers to the nearest

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Received: 19 November 2021 Accepted: 20 January 2022

0.1 mm: SVL, snout-vent length (from the tip of snout to posterior margin of the vent); HDL, head length (from the tip of snout to the articulation of the jaw); HDW, head width (head width at the commissure of the jaws); SNT, snout length (from the tip of snout to the anterior corner of the eye); IND, internasal distance (distance between nares); IOD, interorbital distance (minimum distance between upper eyelids); ED, eye diameter (from the anterior corner of the eye to posterior corner of the eye); TD, tympanum diameter (horizontal diameter of tympanum); TED, tympanum-eye distance (from the anterior edge of tympanum to posterior corner of the eye); HND, hand length (from the proximal border of the outer palmar tubercle to the tip of digit III); RAD, radio–ulna length (from the flexed elbow to the proximal border of the outer palmar tubercle); FTL, foot length (from the distal end of the shank to the tip of digit IV); TIB, tibial length (from the outer surface of the flexed knee to the heel).

The webbing formula follows Savage (1975); tadpole stage was identified following Gosner (1960); sex was determined by examining the nuptial pad. Comparison data were obtained from collected specimens (Appendix 1) and the literature (Boettger, 1895; Boulenger, 1904, 1909; Bourret, 1937; Chang and Hsu, 1932; Chou, 1999; Chuaynkern *et al.*, 2010; Fei *et al.*, 2007, 2009; Kuramoto, 1985; Li *et al.*, 2019; Lyu *et al.*, 2017, 2019, 2020a, 2020b, 2021; Matsui, 2007; Schmidt, 1925; Wei *et al.*, 2020).

2.3. Phylogenetic analysis Maximum likelihood (ML) and Bayesian inference (BI) methods were used to analyze the phylogenetical relationships based on partial 16S ribosomal RNA gene (16S, ~1050 bp) and partial cytochrome oxidase subunit I gene (COI, ~640 bp). We referred to Lyu *et al.* (2017) to isolate and amplify the two mitochondrial DNA fragments. Homologous sequences of *Nidirana* species, mostly from type localities (Table 1), were downloaded from GenBank for phylogenetic analysis. ML was conducted on the CIPRES science gateway with default settings (https://www.phylo.org/portal2/home.action#). BI was implemented using MrBayes v3.2 (Ronquist *et al.*, 2012), and the best-fit model (GTR+I+G) and running parameters following Chen *et al.* (2021).

2.4. Bioacoustics analysis Advertisement calls were recorded in the field at the ambient temperature of 21°C with a SONY ICD-TX50 recorder on 10 May 2021. The software Raven Pro 1.6 (Cornell Laboratory of Ornithology, USA) was used to analyze the call recordings as per Köhler *et al.* (2017).

3. Results

ML and BI analyses of a matrix containing 1775 bp generated identical topologies, consistent with Lyu *et al.* (2021) (Figure 1). Preliminary phylogenetical trees indicate that specimens of SWDS represent a monophyletic group and are closest to *N. guangxiensis* with robust node supports (Figure 1).

Sixteen advertisement calls were recorded in the field. A call group contains 6–8 calls with double notes (Figure 2). Call duration ranges from 1.35 s to 2.11 s (mean 1.66 ± 0.21 s, *n*=16), whereas call interval ranges from 5.68 s to 13.10 s (mean 7.76 ± 1.92 s, *n*=15). The dominant frequencies of double notes are 0.36–2.64 kHz and 0.43–3.25 kHz, respectively. The calls differ from their congeners with regular 6–8 double notes. *Nidirana guangxiensis* calls consist of 6–11 rapidly repeated identical and regular single notes (Lyu *et al.*, 2021) (Figure 2). *Nidirana daunchina, N. lini, N. nankunensis, N. xiangica,* and *N. yeae* issue a significantly different first note (Chou, 1999; Lyu *et al.*, 2017; Lyu and Wang, 2020). *Nidirana adenopleura, N. chapaensis, N. guangdongensis, N. leishanensis, N. occidentalis, N. pleuraden,* and *N. yaoica* have calls without double notes and there are less than six calls in a group (Boulenger 1904; Lyu *et al.*, 2020). *Nidirana hainanensis* issues 2–4 fast-repeated double-notes (Lyu *et al.*, 2017; Li *et al.*, 2019).

Molecular analyses, bioacoustic data, and morphological evidence (see taxonomic account section) indicate that the specimens from SWDS represent a so far unrecognized *Nidirana* species, which is described as follows.

Taxonomic account

***Nidirana shiwandashanensis* sp. nov.** (Figure 3)

Holotype NNU00238, adult male, collected from Shiwandashan National Nature Reserve, Shangsi County, Guangxi, China on 10 May 2021, by WeiCai CHEN.

Paratype NNU00239 and NNU00605, adult males, NNU00606, adult female, collected from the same locality of holotype. NNU00239 collected on 10 May 2021; NNU00605 and NNU00606 collected on 24 August 2021. WeiCai CHEN collected all specimens.

Etymology The species name '*shiwandashanensis*' refers to the type locality, Shiwandashan National Nature Reserve. We suggest the English name as Shiwandashan Music Frog, and the Chinese name as Shi Wan Da Shan Qin Wa (十万大山琴蛙).

Diagnosis *Nidirana shiwandashanensis* **sp. nov.** differs from its congeners in the combination of the following characters: medium body size (SVL 46.2–50.8 mm in males; 48.3 mm in female); dorsal skin smooth without tubercles; ventral surface near immaculate creamy-white without spots; lateroventral grooves present on all fingers and toes; tibiotarsal articulation reaching eye; a call group containing 6–8 calls with double notes.

Description of holotype Adult male, SVL 46.2 mm; head length almost equal to width (HDL/HDW = 1.06); snout oval, significantly protruding beyond lower jaw; canthus rostralis distinct; loreal region sloping; nostril oval and closer to snout

Table 1 Information for samples used in molecular phylogenetic analyses in this study.

ID	Species	Locality (* type locality)	Voucher No.	16S	COI
1	*N. shiwandashanensis* **sp.nov.**	China: Guangxi: Shangsi County*	NNU00238	MZ787977	MZ782098
2	*N. shiwandashanensis* **sp.nov.**	China: Guangxi: Shangsi County*	NNU00239	MZ787978	MZ782099
3	*N. shiwandashanensis* **sp.nov.**	China: Guangxi: Shangsi County*	NNU00605	OK383411	OK377265
4	*N. shiwandashanensis* **sp.nov.**	China: Guangxi: Shangsi County*	NNU00606	OK383412	OK377266
5	*N. shiwandashanensis* **sp.nov.**	China: Guangxi: Shangsi County*	NNU00600	OK383413	OK377267
6	*N. adenopleura*	China: Taiwan: Taichung City	SYS a007358	MN946445	MN945201
7	*N. adenopleura*	China: Taiwan: Taichung City	SYS a007359	MN946446	MN945202
8	*N. chapaensis*	Vietnam: Lao Cai: Sapa*	MNHN 2000.4850	KR827711	KR087625
9	*N. daunchina*	China: Sichuan: Mt Emei*	SYS a004594	MF807822	MF807861
10	*N. daunchina*	China: Sichuan: Mt Emei*	SYS a004595	MF807823	MF807862
11	*N. guangdongensis*	China: Guangdong: Yingde City*	SYS a005767	MN946406	MN945162
12	*N. guangdongensis*	China: Guangdong: Yingde City*	SYS a005768	MN946407	MN945163
13	*N. guangxiensis*	China: Guangxi: Mt Daming*	NHMG 202007001	MZ677222	MZ678729
14	*N. guangxiensis*	China: Guangxi: Mt Daming*	NHMG 202007002	MZ677223	MZ678730
15	*N. hainanensis*	China: Hainan: Mt Diaoluo*	SYS a007669	MN946451	MN945207
16	*N. hainanensis*	China: Hainan: Mt Diaoluo*	SYS a007670	MN946452	MN945208
17	*N. leishanensis*	China: Guizhou: Mt Leigong*	SYS a007908	MN946453	MN945209
18	*N. lini*	China: Yunnan: Jiangcheng County*	SYS a003967	MF807818	MF807857
19	*N. lini*	China: Yunnan: Jiangcheng County*	SYS a003968	MF807819	MF807858
20	*N. mangveni*	China: Zhejiang: Mt Dapan*	SYS a006310	MN946424	MN945180
21	*N. mangveni*	China: Zhejiang: Mt Dapan*	SYS a006311	MN946425	MN945181
22	*N. nankunensis*	China: Guangdong: Mt Nankun*	SYS a005718	MF807839	MF807878
23	*N. nankunensis*	China: Guangdong: Mt Nankun*	SYS a005719	MF807840	MF807879
24	*N. occidentalis*	China: Yunnan: Mt Gaoligong*	SYS a003775	MF807816	MF807855
25	*N. occidentalis*	China: Yunnan: Mt Gaoligong*	SYS a003776	MF807817	MF807856
26	*N. okinavana*	Japan: Okinawa: Iriomote Island*	Not given	NC022872	NC022872
27	*N. pleuraden*	China: Yunnan: Kunming City*	SYS a007858	MT935683	MT932858
28	*N. pleuraden*	China: Yunnan: Wenshan City	SYS a007717	MT935671	MT932850
29	*N. xiangica*	China: Hunan: Mt Dawei*	SYS a006492	MN946434	MN945190
30	*N. yaoica*	China: Guangxi: Mt Dayao*	SYS a007020	MK882276	MK895041
31	*N. yaoica*	China: Guangxi: Mt Dayao*	SYS a007021	MK882277	MK895042
32	*N. yeae*	China: Guizhou: Tongzi County	CIB TZ20190608005	MN295228	MN295234
33	*N. yeae*	China: Guizhou: Tongzi County	CIB TZ20160714016	MN295231	MN295237
34	*Babina holsti*	Japan: Okinawa*	Unknown	NC022870	NC022870
35	*Babina subaspera*	Japan: Kagoshima: Amami Island*	Unknown	NC022871	NC022871

than eye, laterally opening; a creamy white longitudinal ridge present, beginning at posterior of nostril along with upper lip and ending at above insertion of arm; supratympanic fold absent; IOD/IND = 0.68; eye diameter larger than tympanic diameter (ED/TD = 1.36); vomerine teeth oval, closer each other than choana; tongue with a deep notch on posterior; a pair of subgular internal vocal sacs present. Relative finger lengths: II < IV < I < III; tips of fingers slightly dilated with lateroventral grooves; finger webbing and dermal fringes absent; subarticular tubercles prominent and conical; three distinct palmar tubercles; nuptial pad present on finger I. Relative toe lengths: I < II < V < III < IV; tip of toes dilated, forming an arrow-shaped disc with lateroventral grooves; toe webbing formula: I 2 – 2½$^{+}$ II2½$^{-}$ – 3 III 2^{+}– 4½$^{-}$ IV 4 – 3^{-}V; toes with lateral fringes; subarticular tubercles prominent and oval; inner metatarsal tubercles elongated, but outer metatarsal tubercles indistinct; heels not

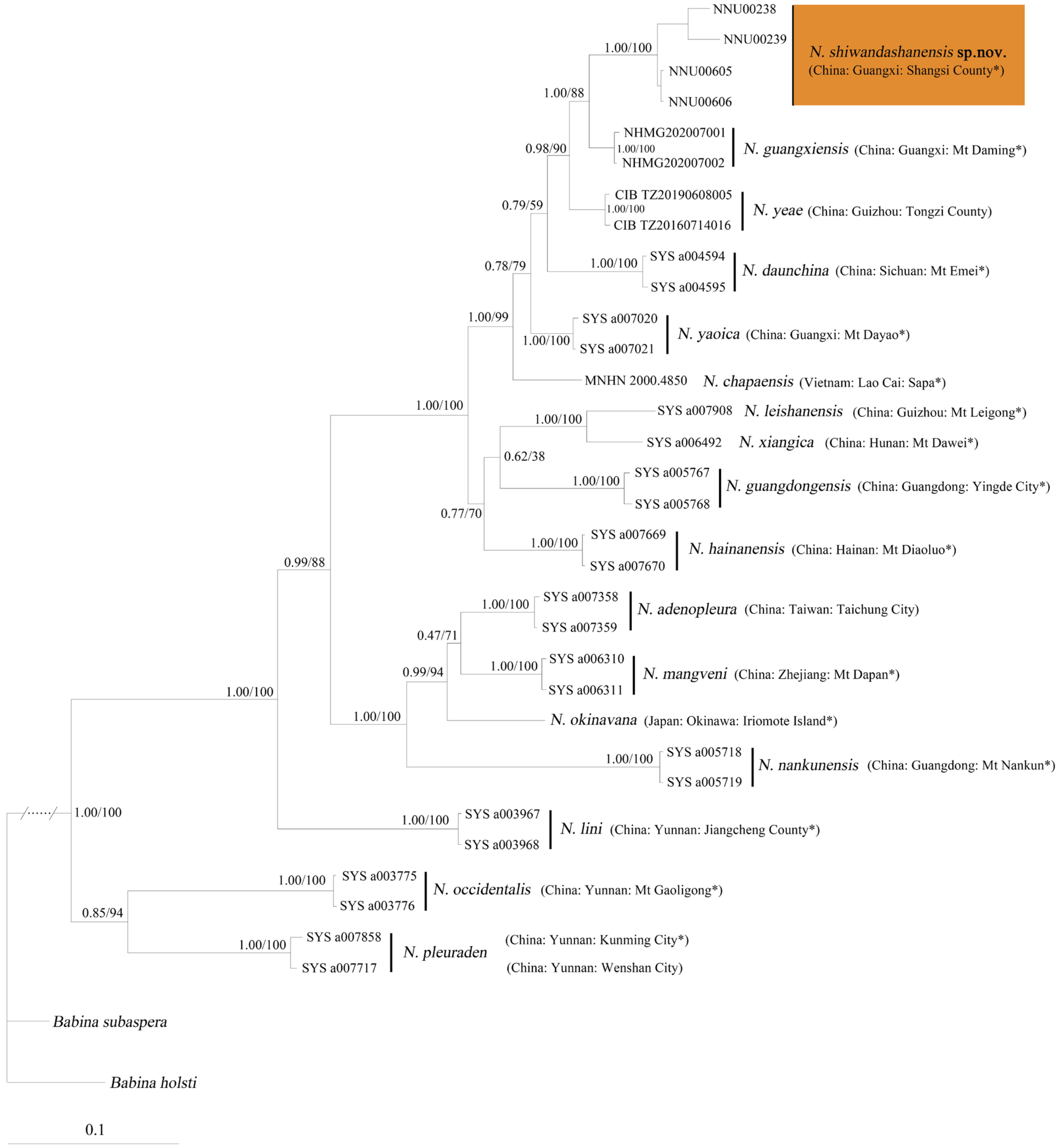
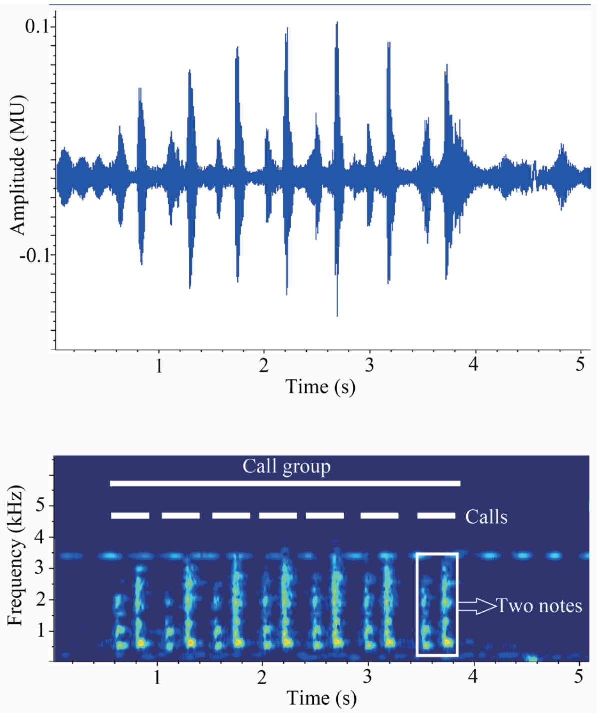

NNU00238
NNU00239
NNU00605
NNU00606
N. shiwandashanensis sp.nov.
(China: Guangxi: Shangsi County*)
1.00/100
1.00/88
NHMG202007001
1.00/100
NHMG202007002
N. guangxiensis (China: Guangxi: Mt Daming*)
0.98/90
CIB TZ20190608005
1.00/100
CIB TZ20160714016
N. yeae (China: Guizhou: Tongzi County)
0.79/59
SYS a004594
1.00/100
SYS a004595
N. daunchina (China: Sichuan: Mt Emei*)
0.78/79
SYS a007020
1.00/99
1.00/100
SYS a007021
N. yaoica (China: Guangxi: Mt Dayao*)
MNHN 2000.4850
N. chapaensis (Vietnam: Lao Cai: Sapa*)
1.00/100
SYS a007908
N. leishanensis (China: Guizhou: Mt Leigong*)
1.00/100
SYS a006492
N. xiangica (China: Hunan: Mt Dawei*)
0.62/38
SYS a005767
1.00/100
SYS a005768
N. guangdongensis (China: Guangdong: Yingde City*)
0.77/70
SYS a007669
1.00/100
SYS a007670
N. hainanensis (China: Hainan: Mt Diaoluo*)
0.99/88
SYS a007358
1.00/100
SYS a007359
N. adenopleura (China: Taiwan: Taichung City)
0.47/71
SYS a006310
0.99/94
1.00/100
SYS a006311
N. mangveni (China: Zhejiang: Mt Dapan*)
1.00/100
1.00/100
N. okinavana (Japan: Okinawa: Iriomote Island*)
SYS a005718
1.00/100
SYS a005719
N. nankunensis (China: Guangdong: Mt Nankun*)
1.00/100
SYS a003967
1.00/100
SYS a003968
N. lini (China: Yunnan: Jiangcheng County*)
SYS a003775
1.00/100
SYS a003776
N. occidentalis (China: Yunnan: Mt Gaoligong*)
0.85/94
SYS a007858
1.00/100
SYS a007717
N. pleuraden
(China: Yunnan: Kunming City*)
(China: Yunnan: Wenshan City)
Babina subaspera
Babina holsti
0.1

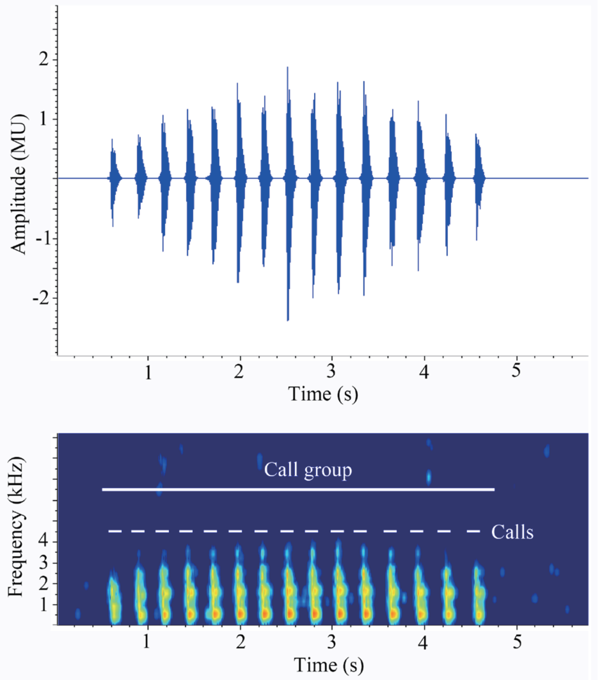
Figure 1 Bayesian Inference tree illustrating phylogenetic relationships among *Nidirana* based on the mitochondrial 16S and COI genes. Numbers at nodes are posterior probabilities (left) and maximum likelihood bootstrap values (right) from a separate Maximum likelihood analysis of the same dataset. * represents type locality.

meeting when thighs held at right angles to body; tibiotarsal articulation reaching eye. Dorsum smooth without tubercles on posterior; hindlimbs with several longitudinal dermal ridges; dorsolateral fold beginning at posterior of eye and ending at above groin and irregular small glands present along its inferior margin; pineal gland distinct; flanks with a single gland each; vent with some small tubercles around (Figure 3).

Color of holotype In life, dorsum light brown with several sparse black spots; pineal gland creamy-white; dorsum medially with a creamy-white line, beginning at pineal gland and ending



Nidirana shiwandashanensis **sp. nov.**



Nidirana guangxiensis

Figure 2 Advertisement calls of *Nidirana shiwandashanensis* **sp. nov.** and *N. guangxiensis* (NNU00213) from the type localities.

at vent; presence of a black line beginning at tip of snout, along the inferior margin of dorsolateral fold and ending at groin; tympanum brown with a black marking at posterior; presence of a creamy-white linear gland on upper jaw; maxillary gland creamy-white; flanks with several black spots and a large greyish-yellow glandular area; thigh and tibia with three grey bands; ventral surfaces of throat, chest, belly, and thigh near immaculate creamy white; anterior of base of forelimb with a dark stripe. In preservation, dorsal surface fading to deep grey; black spots displaying darkish black.

Variations Measurements of type series are listed in Table 2. Paratypes are similar to holotype in morphology and color pattern. The colorations of transverse bands on thigh and tibia vary from grey to dark. Spots on dorsal surface of thigh are variable, some having more dense spots than others. Black spots on dorsum vary too. Female individual (NNU00606) lacks large greyish-yellow glandular areas on flanks.

Tadpole In the 40th stage (NNU00600), body length 16.8 mm, tail length 33.9 mm, maximal tail height 10.2 mm, eyes lateral, spiracle sinistral, dorsoposterior directed, dorsal fin arising at the base of the tail, labial tooth row formula: I: 1+1/1+1: II (Figure 3F). In life, body brown, tail pale brown with pigmented tiny spots; body and tail grey in preservation.

Ecology and distribution *Nidirana shiwandashanensis* **sp. nov.** was found in the swamp at evergreen forests between 500–600 m a.s.l., where there was no running water. On 10 May 2021, we heard the advertisement calls in the field but have not found eggs. On 24 August 2021, we revisited the same site and found the tadpole in 40th stage and the sub-adult specimen (Figure 3G, H). We assume that the breeding season ranges from 5 to 7 months. We did not observe the new species has nest construction behavior. So far, we only found this new species in SWDS. Whether it also occurs in the adjacent reserve, Golden Camellia National Nature Reserve, or has an even larger actual range, had to be determined through further fieldwork.

Comparison A summary of morphological characters is provided in Table S1. *Nidirana shiwandashanensis* **sp. nov.** differs from its congeners based on the following characters: (1) SVL 46.2–50.8 mm in males; (2) dorsal skin smooth without tubercles; (3) ventral surface near immaculate creamy-white; (4) tibiotarsal articulation reaching eye; (5) relative finger length II < IV < I < III; (6) lateroventral grooves present on all fingers and toes; (7) nuptial pad present on Finger I; (8) a call containing double notes.

Phylogenetically, *N. shiwandashanensis* **sp. nov.** is closest to *N. guangxiensis* (Figure 2). However, *N. shiwandashanensis* **sp. nov.** differs from *N. guangxiensis* by relative fingers length II < IV < I < III (vs. II < I < IV < III); tibiotarsal articulation reaching eye (vs. tibiotarsal articulation reaching nostril); absent dermal fringes on fingers (vs. broad lateral fringes on inner sides of Fingers II, III and IV but absent on Finger I); lateroventral grooves present on all fingers (vs. lateroventral grooves present on Fingers III and IV); toe webbing formula: I 2 – 2½$^{+}$ II2½$^{-}$ – 3 III 2^{+}– 4½$^{-}$ IV 4 – 3^{-}V (vs. I 1⅓–2 II 1⅓–2⅓ III 1⅔–3 IV 3⅓–1⅓ V); smooth

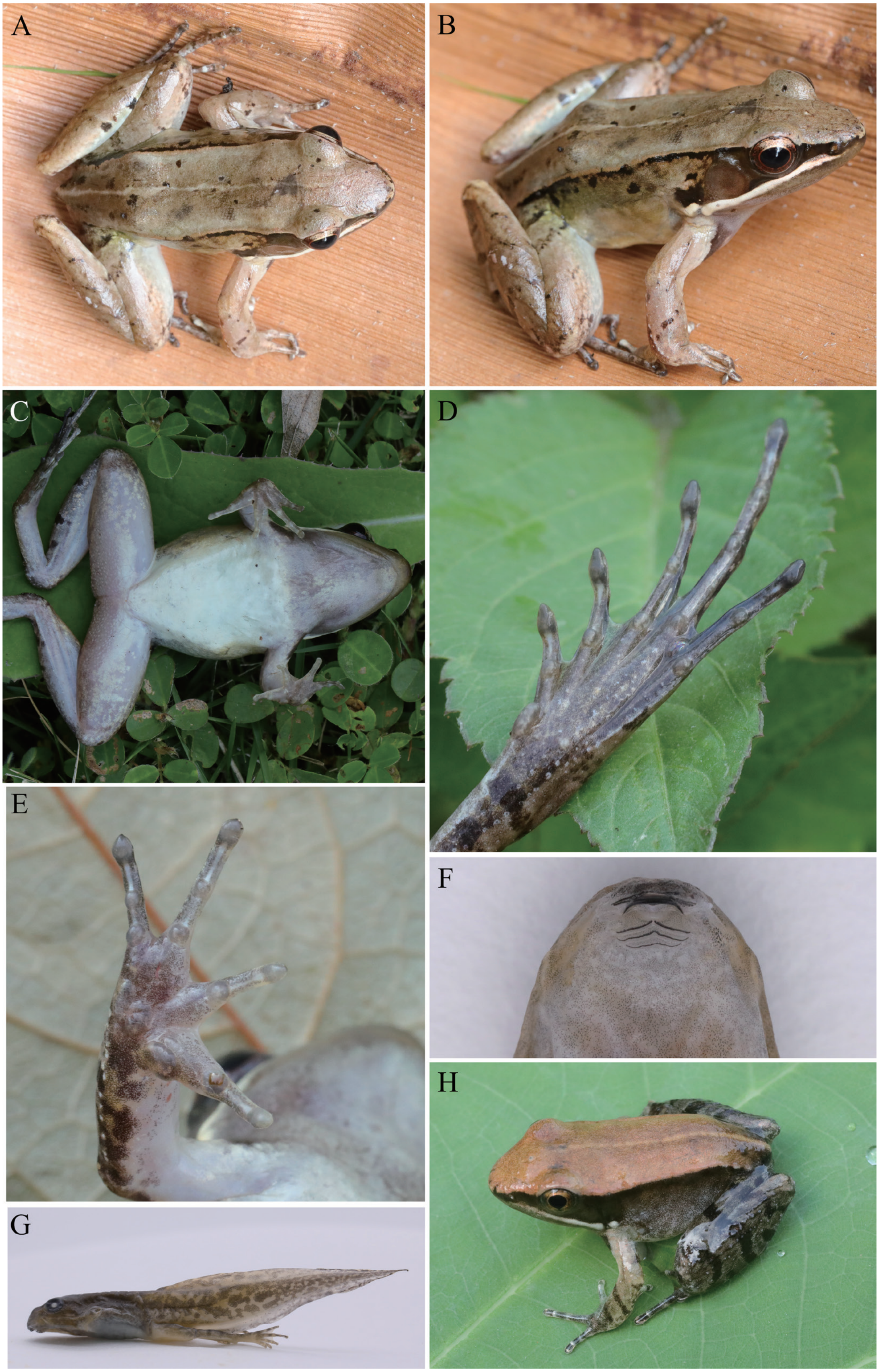


Figure 3 A–H: *Nidirana shiwandashanensis* **sp. nov.** A: dorsal view; B: lateral view; C: ventral view; D: ventral view of foot; E: ventral view of hand; F: labial tooth row formula; G: tadpole in the 40^{th} stage; H: sub-adult.

Table 2 Measurements of *Nidirana shiwandashanensis* **sp. nov.** (in mm). Abbreviations defined in Materials and Methods.

	NNU00238 (Male)	NNU00239 (Male)	NNU00605 (Male)	NNU00606 (Female)
SVL	46.2	50.8	49.2	48.3
HDL	17.6	17.3	17.3	17.1
HDW	16.7	16.3	16.9	15.6
SNT	6.6	7.4	7.4	7.1
IND	6.3	6.8	6.3	6.4
IOD	4.3	5.6	5.2	4.8
ED	5.9	5.8	5.2	5.8
TD	4.3	5.1	4.8	4.4
TED	1.5	1.3	1.5	1.4
HND	10.9	11.7	12.6	11.5
RAD	8.5	8.3	8.4	8.1
FTL	24	24	25.3	25.7
TIB	25	24.5	24.4	24.3

dorsal skin without tubercles (vs. rough dorsal skin with dense granules); a call containing 6–8 double notes (vs. 6–11 rapidly repeated identical and regular notes) (Figure 2).

By its medium body size (SVL 46.2–50.8 mm in males), *N. shiwandashanensis* **sp. nov.** differs from *N. nankunensis* (vs. SVL < 38 mm in males); by relative finger lengths II < IV < I < III, *N. shiwandashanensis* **sp. nov.** differs from *N. leishanensis* and *N. yeae* (vs. II < IV < I < III), *N. chapaensis* (II < I = IV < III), and *N. mangveni* (vs. I < II < IV < III); by lateroventral grooves present on all fingers (vs. absent *N. yeae, N. occidentalis* and *N. pleuraden*; present all but finger I *N. chapaensis, N. adenopleura, N. guangdongensis, N. lini, N. nankunensis,* and *N. okinavana*); by lateroventral grooves present on all toes (vs. absent on all toes in *N. occidentalis* and *N. pleuraden*); by tibiotarsal articulation reaching eyes (vs. beyond the tip of snout in *N. lini*; at nostril in *N. adenopleura, N. chapaensis, N. daunchina, N. guangdongensis, N. hainanensis, N. nankunensis, N. pleuraden*, and *N. yaoica*); by spinules absent on posterior dorsal skin (vs. present in *N. adenopleura, N. lini, N. mangveni, N. occidentalis, N. pleuraden,* and *N. xiangica*); by single nuptial pad present on Finger I (vs. absent in *N. hainanensis*; two part on Finger I in *N. chapaensis*; present on Fingers I and II in *N. leishanensis*) (Table S1).

4. Discussion

Comprehensive analyses, including morphology, DNA sequences, and bioacoustics, strongly support identifying the herein described specimens from SWDS as a new species in the genus *Nidirana*. Based on the mtDNA analysis, *N. shiwandashanensis* **sp. nov.** and *N. guangxiensis* are sister taxa. Although the two species have similar morphological characters, *N. shiwandashanensis* **sp. nov.** and *N. guangxiensis* display clearly distinct bioacoustics and nest construction behavior. The advertisement calls of the new species consist of 6–8 calls with double notes, whereas the calls of *N. guangxiensis* contain 6–11 rapidly repeated identical and regular single notes (Figure 2). Additionally, *N. shiwandashanensis* **sp. nov.** has not to nest construction behavior, other than *N. guangxiensis.*

Up to date, there are five recognized *Nidirana* species in Guangxi, displaying impressive species diversity. Our discovery of *N. shiwandashanensis* **sp. nov.** demonstrates that previous studies treating Guangxi *Nidirana* populations solely as *N. adenopleura* were erroneous (Liu and Hu, 1962; Fei *et al.*, 2009; Mo *et al.*, 2014; Zhang and Wen, 2000). Lyu *et al.* (2021) and our results both indicate that *Nidirana* species have relatively narrow ranges and that rivers, and mountains contribute to the speciation of *Nidirana* species. For instance, Xijiang Rive separates *N. shiwandashanensis* **sp. nov.** and *N. guangxiensis*, whereas Hongshuihe Rive separates *N. guangxiensis* and *N. yaoica*. Nowadays, no *N. adenopleura* have been found in northern, southern, eastern, and central Guangxi. Whether *N. adenopleura* occurs in western Guangxi needs to be investigated further.

Acknowledgments The authors are grateful to the staff of Shiwandashan National Nature Reserve for assistance during fieldwork and two anonymous reviewers for improving the manuscript. Additionally, the authors would like to express their gratitude to EditSprings (https://www.editsprings.cn/) for the expert linguistic services provided. This work was supported by the National Natural Science Foundation of China (32060116) and Guangxi Natural Science Foundation, China (2020GXNSFDA238022).

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Handling Editor: Heling Zhao

How to cite this article:

CHEN W. C., PENG W. X., LI P., LIU Y. J. A New Species of *Nidirana* (Anura, Ranidae) from Southern Guangxi, China. Asian Herpetol Res, 2022, 13(2): 109–116. DOI: 10.16373/j.cnki.ahr.210065

Appendix

Table S1 Diagnostic characters separating *Nidirana shiwandashanensis* **sp. nov.** from all congeners.

ID	Species	SVL of males (mm)	SVL of females (mm)	Fingers tips	Lateroventral groove on fingers	Relative length of fingers	Toes tips	Lateroventral groove on toes	Tibiotarsal articulation	Subgular vocal sacs	Nuptial pad	Spinules on dorsal skin	Nest construction	Tadpole labial tooth row formula	Calling	References
1	*N. shiwandashanensis* **sp. nov.**	46.2-50.8	48.3	Dilated	Present	II < IV < I < III	Dilated	Present	Eye	Present	One on finger I	Absent	Absent	I: 1+1/1+1:II	6–8 double notes	This study
2	*N. adenopleura*	43.1–57.6	47.6–60.7	Dilated	Present except finger I	II < I < IV < III	Dilated	Present	Snout tip or eye-snout	Present	One on finger I	Entire or posterior	Absent	I:1+1/1+1:II or I:0+0/1+1:I	2–5 regular notes	Lyu *et al.* (2017, 2020b)
3	*N. chapaensis*	35.5–42.5	41.0–51.8	Dilated	Present except finger I	II < I = IV < III	Dilated	Present	Nostril	Present	Two on finger I	Absent or few above vent	Present	I:1+2/1+1:II	3 notes	Chuaynkern *et al.* (2010)
4	*N. daunchina*	40.6–51.0	44.0–53.0	Dilated	Absent or rarely present	II < I < IV < III	Dilated	Present	Nostril	Present	One on finger I	Absent	Present	I:1+1/1+1:II or I:1+1/2+2:I	2–5 notes containing a specific first note	Liu (1950); Lyu *et al.* (2017)
5	*N. guangdongensis*	50.0–58.4	55.3–59.3	Dilated	Present except finger I	II < I < IV < III	Dilated	Present	Nostril	Present	One on finger I	Entire	Absent	?	2–4 regular notes	Lyu *et al.* (2020b)
6	*N. guangxiensis*	40.2–47.6	49.9–51.0	Dilated	Present on fingers III and IV	II < I < IV < III	Dilated	Present	Nostril	Present	One on finger I	Absent	Present	I: 1+1/1+1:II	6–11 rapidly repeated regular notes	Lyu *et al.* (2021)
7	*N. hainanensis*	32.8–44.4	?	Dilated	Present	II < I < IV < III	Dilated	Present	Nostril	Present	Absent	Absent	Present	?	2–4 fast-repeated double-notes	Fei *et al.* (2009, 2012)
8	*N. leishanensis*	49.5–56.4	43.7–55.3	Dilated	Present	II < IV < I < III	Dilated	Present	Eye-snout	Present	Two on fingers I and II	Absent	Absent	I:1+2/ 1+1:II	1 single note	Li *et al.* (2019)
9	*N. lini*	44.1–63.1	57.7–68.6	Dilated	Present except finger I	II < I < IV < III	Dilated	Present	Beyond snout	Present	One on finger I	Posterior	Absent	I:1+1/1+1:II	5–7 notes containing a specific first note	Chou (1999); Lyu *et al.* (2017)
10	*N. mangveni*	53.6–59.7	59.7–65.1	Dilated	Present on fingers III and IV	I < II < IV < III	Dilated	Present	Anterior corner of eye	Present	One on finger I	Entire or posterior	Absent	?	2–7 regular notes	Lyu *et al.* (2020b)
11	*N. nankunensis*	33.3–37.1	37.8–39.5	Dilated	Present except finger I	II < I < IV < III	Dilated	Present	Nostril	Present	One on finger I	Absent or few above vent	Present	I:1+1/1+1:II	13–15 notes containing a specific first note	Lyu *et al.* (2017)
12	*N. occidentalis*	44.5–53.0	55.6–61.3	Not dilated	Absent	II < I < IV < III	Not dilated	Absent	Eye	Present	One on finger I	Posterior	Absent	?	3–5 regular notes	Lyu *et al.* (2020a)
13	*N. okinavana*	35.5–42.8	44.6–48.8	Dilated	Present except finger I	II < I < IV < III	Dilated	Present	Eye center-near nostril	Absent	Poorly one on finger I	Absent	Present	I:1+1/1+1:II	10–25 fast-repeated notes	Chuaynkern *et al.* (2010); Lyu *et al.* (2017)
14	*N. pleuraden*	46.2–52.3	46.9–61.7	Not dilated	Absent	II < I < IV < III	Not dilated	Absent	Nostril	Present	One on finger I	Posterior	Absent	I:1+1/1+1: II	1–4 regular notes	Lyu *et al.* (2017, 2020a)
15	*N. xiangica*	56.3–62.3	53.5–62.6	Dilated	Present	II < I < IV < III	Dilated	Present	Eye-snout	Present	One on finger I	Entire	Absent	?	2–3 notes containing a specific first note	Lyu *et al.* (2020b)
16	*N. yaoica*	42.1–45.6	?	Dilated	Present	II < I < IV < III	Dilated	Present	Nostril	Present	One on finger I	Absent	? (Probably present)	?	1–3 fast-repeated regular notes	Lyu *et al.* (2019)
17	*N. yeae*	41.2–43.5	44.7	Dilated	Absent	II < IV < I < III	Dilated	Present	Eye	Present	One on finger I	Absent	? (Probably absent)	I: 1+1/1+1:II	2–6 notes containing a specific first note	Wei *et al.* (2020)